

Interleukin-1 β and tumour necrosis factor- α levels in periapical exudates

T. Ataoğlu¹, M. Üngör², B. Serpek⁴, S. Haliloğlu⁴, H. Ataoğlu³ & H. Ari²

Departments of ¹Periodontology, ²Endodontics, and ³Maxillofacial Surgery, Dentistry Faculty; and ⁴Department of Biochemistry, Veterinary Faculty, Selcuk University, Konya, Turkey

Abstract

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Aim The aim of this study was to determine IL-1 β and TNF- α levels in periapical exudates and to evaluate their relationship with clinical and radiological findings.

Methodology Periapical exudates were collected from root canals of 35 single-rooted teeth using absorbent paper points. IL-1 β and TNF- α levels were determined by enzyme-linked immunosorbent assays. The samples were divided into two groups according to the presence or absence of clinical signs including swelling and/or fistula, pain on palpation and/or percussion, and pus discharge from canals. Periapical exudate samples were

divided into two groups according to size of periapical radiolucent area.

Results The mean concentration of IL-1 β (72.79 ng mL⁻¹) in periapical exudates was approximately 12-fold higher than TNF- α (6.17 ng mL⁻¹). There was no significant correlation between these cytokines ($P > 0.05$). IL-1 β levels in canals with larger radiolucent areas (long axis ≥ 1 cm) were significantly higher than those with small areas ($P < 0.05$). There was a tendency toward higher levels of IL-1 β in groups with clinical signs, but the differences were not significant ($P > 0.05$).

Conclusions Periapical exudate levels of both cytokines failed to reflect periapical disease state.

Keywords: interleukin-1 β , periapical diseases, periapical granuloma, tumour necrosis factor- α .

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Introduction

It is well known that bacterial infection of the dental pulp results in the formation of periapical lesions and causes the destruction of bone in the periapical region. The presence of immunocompetent cells such as macrophages, T and B cells and plasma cells has been demonstrated in human periapical lesions, suggesting that the host immune response is involved in the pathogenesis of the disease (Stern *et al.* 1981, Matthews & Mason 1983, Torabinejad & Kettering 1985, Torabinejad *et al.* 1985).

Cytokines such as interleukin-1 β (IL-1 β), interleukin-1 α tumour necrosis factor- α (TNF- α) are soluble mediators and released from immunocompetent cells in inflammatory processes. These cytokines stimulate

bone resorption, prostaglandin synthesis and protease production by many cell types, including fibroblasts and osteoblasts (Dewhirst *et al.* 1985, Beutler & Cerami 1986, Billingham 1987, Lorenzo *et al.* 1987, Tatakis *et al.* 1988). The local production of PGE₂, IL-1 β and TNF- α has been shown in periapical lesions (Artese *et al.* 1991, McNicholas *et al.* 1991, Shimauchi *et al.* 1998).

Exudate is a product of the inflammatory response that contains host mediators associated with the response. In previous reports, periapical exudate collected from root canals have been used to study host responses in endodontic lesions and detectable levels of IL-1 β , IL-1 α , PGE₂ and TNF- α in periapical exudates were demonstrated (Safavi & Rossomando 1991, Matsuo *et al.* 1994, Takayama *et al.* 1996). Reports attempting to correlate the levels of cytokines and clinical signs in involved teeth are rare and the results are conflicting. In this regard, the present study aimed to determine IL-1 β and TNF- α levels in periapical exudates and to evaluate the possible

Correspondence: Dr. Tamer Ataoğlu, Selcuk Universitesi, Dishekimligi Fakultesi, Periodontoloji Anabilim Dalı 42079 Kampus, Konya, Turkey (fax: +332 241 00 62; e-mail: ataoglu@selcuk.edu.tr).

relationships between these cytokines and clinical signs in the involved teeth.

Materials and methods

Subjects

Patients scheduled to undergo root canal treatment because of periapical lesions were selected for the study. The aim of the study was explained to patients and consent was obtained. The medical histories of all patients were non-contributory. Samples of periapical exudates were obtained from root canals of 35 single-rooted teeth with radiolucent areas around the periapex. None of the lesions were related to marginal periodontitis and none extended to the maxillary sinuses.

Clinical findings

Clinical signs associated with the involved teeth, including swelling, fistula on the periapical mucosa, tenderness to percussion, pain on palpation of the periapical mucosa and pus discharging in the canal, were evaluated and recorded at the sampling visit. Pus discharge was judged by the colour, transparency and viscosity of the exudate. For comparisons of cytokine levels in periapical exudates, teeth were divided into two groups according to presence or absence of clinical signs, including pain on percussion and/or on palpation of overlying mucosa, swelling and/or fistula, and pus discharge from the canals. A radiographic examination was completed at the time of sampling. The paralleling technique or the bisecting-angle technique were used. Sizes of radiolucent areas around the periapex were determined by two examiners. Periapical lesions were classified as small when the long axis of the radiolucent area was shorter than 1 cm and all the others as large. Periapical exudate cytokine levels of teeth with large and small lesions were also compared.

Sampling of periapical exudates

The involved teeth were isolated with a rubber dam. Penetration to the pulp chamber and unroofing were made by low-speed round burs as preparations for entering the chamber. The tooth was accessed carefully, without contamination by water or irrigants. Following the measurement of the working length, the root canal was enlarged to size 40. After the root canal was dried with sterilized paper points, two size 40 paper points (Absorbent paper points, Kerr Manufacturing Co., Romulus, MI, USA) were

subsequently inserted into the root canal close to the established working length and held for 30 s. In actively draining teeth, paper points were taken out immediately or left for a few s in the canals. If the paper point withdrawn from the canal was dry, a thin endodontic file was used carefully to penetrate through the apical foramen to bring exudate into the root canal from the periapical area. Blood was occasionally seen in the suppurative exudates. The tooth was excluded from the study when more than a small amount of visible blood was present. The wetted length of paper points was measured immediately. The volume of the fluid was calculated from a standard curve as described before (Shimauchi *et al.*, 1996) and expressed as μL . Both paper points were placed in a tube containing 250 μL of PBS, vortexed for 1 min and stored at -30°C until time of assay. Routine treatment of the patient was then continued.

Measurement of IL-1 β and TNF- α levels

IL-1 β and TNF- α were measured by enzyme-linked immunosorbent assays. Assays were carried out in accordance with manufacturer's instructions (Immunotech, Marseille, France). The amount of IL-1 β and TNF- α were determined by reference to standard curves ($0\text{--}1000\text{ pg mL}^{-1}$) constructed with each assay. The concentrations of IL-1 β and TNF- α in each sample were calculated based on the dilutions and exudate volumes. The results were expressed as nanograms per milliliter (ng mL^{-1}) for cytokine concentration.

Data analysis

IL-1 β and TNF- α levels in the periapical exudates were compared between groups by using Mann-Whitney *U*-test. The relationships between cytokine levels and exudate volumes were determined by using Pearson correlation coefficients.

Results

IL-1 β was detected in all periapical exudate samples, whereas TNF- α was found in 25 of 35 samples. IL-1 β and TNF- α concentrations were in wide ranges and IL-1 β was present in approximately 12-fold higher concentrations than TNF- α (Table 1).

The exudates from the canals with larger radiolucent areas contained significantly higher IL-1 β than those from the canals with small radiolucent areas ($P < 0.05$) (Table 2). No significant difference in the IL-1 β and TNF- α levels was found when the exudates were divided by the

Table 1 Descriptive statistical data

		Mean (ng mL ⁻¹)	SD	Minimum	Maximum
<i>Radiolucent area</i>					
≥ 1 cm ($n = 17$)	IL-1 β	117.12	147.38	1.52	502.35
	TNF- α	4.51	8.25	0.00	32.51
< 1 cm ($n = 18$)	IL-1 β	30.92	35.89	0.63	135.74
	TNF- α	7.74	9.37	0.00	30.38
<i>Pain (palpation/percussion)</i>					
Present ($n = 12$)	IL-1 β	83.84	141.89	1.52	502.35
	TNF- α	2.65	4.44	0.00	12.75
Absent ($n = 23$)	IL-1 β	67.03	97.86	0.63	429.11
	TNF- α	8.01	10.06	0.00	32.51
<i>Pus discharge</i>					
Present ($n = 6$)	IL-1 β	146.02	184.91	6.45	502.35
	TNF- α	1.95	2.45	0.00	6.33
Absent ($n = 29$)	IL-1 β	57.64	89.56	0.63	429.11
	TNF- α	7.05	9.47	0.00	32.51
<i>Swelling/fistula</i>					
Present ($n = 16$)	IL-1 β	71.94	124.10	1.52	502.35
	TNF- α	6.15	9.60	0.00	30.38
Absent ($n = 19$)	IL-1 β	73.51	106.28	0.63	429.11
	TNF- α	6.19	8.46	0.00	32.51
<i>Total samples</i>					
($n = 35$)	IL-1 β	72.79	113.03	0.63	502.35
	TNF- α	6.17	8.86	0.00	32.51

Table 2 Comparisons among groups regarding cytokine levels

	Mean Rank	Mean Rank	U	Z	2-tailed <i>P</i>
<i>Radiolucent area</i>					
≥ 1 cm		< 1 cm			
IL-1 β	21.85	14.36	87.5	-2.162	0.031*
TNF- α	15.38	20.47	108.5	-1.486	0.137
<i>Pain (palpation/percussion)</i>					
Present		Absent			
IL-1 β	18.29	17.85	134.5	-0.122	0.903
TNF- α	13.46	20.37	83.5	-1.916	0.055
<i>Pus discharge</i>					
Present		Absent			
IL-1 β	23.50	16.86	54.0	-1.444	0.149
TNF- α	13.83	18.86	62.0	-1.107	0.268
<i>Swelling/fistula</i>					
Present		Absent			
IL-1 β	17.00	18.84	136.0	-0.530	0.596
TNF- α	17.16	18.71	138.5	-0.452	0.651

*Mann-Whitney *U*-test, significant ($P < 0.05$).**Table 3** Correlations between periapical exudate volume and IL-1 β /TNF- α concentrations

	<i>r</i>	<i>P</i>
IL-1 β – TNF- α	-0.140	0.423
Exudate volume – TNF- α	-0.342	0.044*
Exudate volume – IL-1 β	0.195	0.261

*Simple correlation analysis – Pearson correlation coefficient, significant ($P < 0.05$).

reaction to palpation of overlying mucosa and/or to percussion, the presence of pus discharge from the canals, or the presence of swelling and/or fistula ($P > 0.05$). There was a statistically significant, but weak negative correlation between TNF- α and exudate volume ($P < 0.05$) (Table 3).

Discussion

Although periapical granulomas occur commonly, the pathological mechanisms that are responsible for their

expansion have not been fully established. Periapical bone resorption is a prominent feature of periapical lesion development and various kinds of inflammatory mediators, including IL-1 β and TNF- α and other bone resorptive cytokines, were determined in the exudate of periapical lesions (Matsuo *et al.* 1994, Takayama *et al.* 1996). From *in vitro* studies, IL-1 β has been found to be 500-fold more potent than TNF- α in mediating bone resorption and constitutes the major component of osteoclast activating factor (Stashenko *et al.* 1987, Stashenko *et al.* 1989). Although these findings indicate that IL-1 β may be more relevant with respect to periapical bone resorption, the relative concentration of TNF- α actually present in periapical exudate has not yet been determined. In this respect, by immunoassay of periapical exudates from canals, we determined IL-1 β and TNF- α levels.

Both cytokines, IL-1 β and TNF- α are mainly produced by cells belonging to the mononuclear phagocyte system and cells positively stained for IL-1 β and TNF- α have been demonstrated in periapical granulomas (Artese *et al.* 1991). In the present study, IL-1 β was detected in all of the periapical exudate samples, whereas TNF- α was found in 25 of 35 samples. IL-1 β was present in approximately 12-fold greater quantities than TNF- α . Furthermore, IL-1 β levels were significantly higher in periapical exudates of teeth with larger radiolucent areas. Therefore, on the basis of greater quantity in periapical exudate of larger lesions, as well as potency in stimulating bone resorption, we conclude that of these mediators, IL-1 β is likely to be of greater importance in periapical bone loss.

In studies attempting to correlate IL-1 β levels and clinical findings, conflicting results have been reported. Lim *et al.* (1994) demonstrated that symptomatic human periradicular lesions showed a trend to contain more IL-1 β levels than asymptomatic lesions. Matsuo *et al.* (1994) could not find any significant difference of IL-1 β levels when the exudates were divided into two groups according to the presence of similar clinical findings used in this study. Kuo *et al.* (1998) have reported higher concentrations of IL-1 β in the exudates collected from teeth with suppuration. In this study, we could not determine significantly higher IL-1 β levels when the clinical findings were present. Pus discharging canals contained approximately 3-fold higher IL-1 β levels than non-discharging canals. However, the difference between groups was not statistically significant, probably due to our small sample size for pus discharging canals and distribution of data in wide range.

Detectable levels of TNF- α levels in the periapical exudates were determined by Safavi & Rossomando

(1991). Artese *et al.* (1991) located cells stained positively for TNF- α in periapical granulomas, suggesting its bone resorptive role in the pathogenesis of periapical granulomas. To our knowledge, this is the first study evaluating the association between periapical exudate TNF- α levels and clinical findings. Data obtained from this study revealed that TNF- α levels were relatively low when compared to IL-1 β levels and there was no correlation between these cytokines. Interestingly, TNF- α levels negatively correlated with exudate volume and tended to be lower in the presence of clinical findings. These findings suggest that TNF- α levels in the periapical exudates are not reflecting inflammatory conditions. In a tissue culture study, Stashenko *et al.* (1987) have demonstrated a synergistic interaction between IL-1 β and TNF- α in bone resorbing activity. Nevertheless, we are not able to determine such a synergy and our results suggest that, at least, it is not operative in periapical bone resorption.

Conclusions

It is apparent that the progression of periapical granulomas is accompanied by the rate at which their surrounding bone is destroyed. The results of the present study suggest that IL-1 β is more important than TNF- α in periapical bone resorbing activity. Within the limitations of this study, it is concluded that periapical exudate levels of both cytokine are not able to reflect periapical disease state.

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